

## Research Article

# Role of C-Reactive Protein in the Development of Atherosclerosis in Diet-induced Lipidemia in Albino Rats

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## Abstract

**Purpose:** Blood C-reactive protein level serves as a useful biomarker of generalized injury and inflammation. Vast amounts of clinical data suggest its serum level predicts risk of cardiovascular disease. In the present study, the role of C-reactive protein in the development of atherosclerosis in albino rats was investigated.

**Methods:** Wistar breed albino rats were divided into two groups. Group I was fed on normal pellet diet while group II fed on high fat diet for up to 120 days, and blood serum was analyzed for C-reactive protein and lipid profile. Lipid profile included determination of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides. After sacrifice of the animals, the heart and aorta were removed for histopathological studies.

**Results:** The concentration of C-reactive protein did not increase in group II but a significant increase in lipid profile was observed ( $p < 0.001$ ). In histopathological studies, sections of the aorta showed lesions in tunica intima; the branch of coronary artery showed lipid infiltration in its wall while myocardial infarct was clearly observed in the myocardium.

**Conclusion:** Feeding the rats high fat diet for 120 days did not increase C-reactive protein level but significant increases in lipid levels were observed in group II. These data indicate that in albino rats, C-reactive protein does not play any role in the development of atherosclerosis.

**Keywords:** C-reactive protein, Hyperlipidemia, Coronary Heart Disease.

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## INTRODUCTION

There is increasing evidence that complement activation may play an important role in atherogenesis. Thus activation products of the complement cascade have been demonstrated in atherosclerotic lesions of experimental animals and humans. C-reactive protein (CRP) is the prototype acute-phase protein in humans and numerous animals and it is widely used as an indicator of the activity of bacterial infection and various other diseases. In the acute-phase response, its plasma concentration can be elevated up to 500 times its normal level [1]. It is a marker of inflammation, and is synthesized mainly in the liver and regulated by circulating interleukin (IL-6).

Emerging evidence suggests that elevated plasma levels of CRP have become one of the strongest independent predictors of coronary heart disease [2,3]. Mild elevations in CRP even when within the clinically 'normal' range are independently predictive of future cardiovascular events [4,5]. CRP directly participates in the process of atherogenesis by modulating endothelial function and its concentration known to predict cardiovascular events [6]. Recently, elevated level of CRP has been associated with the feature of the insulin resistance syndrome [7]. For clinical purposes, it is the most promising inflammatory biomarker, a classical acute-phase marker and a member of the pentraxin family of innate immune response protein [8]. It has a long half-life, affording stability of levels with no observable circadian variation [9]. Further, it is easily measured in usual outpatient settings, and standardized high sensitivity assays commercially available provide similar results in fresh, stored, and frozen plasma [10].

In this study, atherosclerosis was induced in animals using high fat diet. The main objective was to assess the role of CRP in the development atherosclerosis in albino rats.

## EXPERIMENTAL

### Animals

Adult male albino rats of Wistar strain, weighing between 180 and 250 g, were used for the study. They were procured from the National Institute of Nutrition, Hyderabad, India. Approval of the institutional animal ethical committee was obtained for the animal studies and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines were followed throughout the experiment. The animals were housed in polypropylene cages at  $25 \pm 2^{\circ}$  C and 12h/12h light/dark cycle, and fed with standard pellet diet and water *ad libitum*. The animals were divided into two groups of 12 rats each. Group I rats served as control and were fed normal pellet diet. Group II was fed on high fat diet (HFD) for 120 days to develop atherosclerosis. The composition of high fat diet is depicted in Table 1.

**Table 1:** Composition of high fat diet (HFD) for rats

Ingredient	%w/w
Cholesterol	5.0
Salt mixture	4.0
Casein	20.0
Cellulose	6.0
Choline chloride	0.2
Sugar	25.0
Butter	40.0

Next, i.e., after 120 days, the rats were fasted for 12 h before collection of blood samples. Each rat was anesthetized with diethyl ether and blood was withdrawn from the retro-orbital plexus, transferred to a centrifuge tube and allowed to clot. The separated serum was assayed for C-reactive protein by latex agglutination method [11] using Accucare c-reactive protein reagent latex test diagnostic kit (Labcare Diagnostics India Pvt Ltd). Total

cholesterol, HDL, VLDL and LDL level were assayed using Span diagnostic kits.

### Histopathological studies

The animals were sacrificed, the heart and aorta were removed as quickly as possible, fixed in 10 % formalin and prepared for histological examination [12]. The tissues were embedded in gelatin to prepare frozen sections using microtome. The aortae were first stained (gross staining) and examined for lesions with the aid of a magnifying lens. They were then cut longitudinally on the microtome, mounted and examined for lesions under a light microscope. The frozen sections of the aortae were stained with oil red O in triethylphosphate. Heart and frozen sections of the tissue were embedded in paraffin and stained routinely with hematoxylin and eosin [13-15].

### Statistical analysis

Data are expressed as mean  $\pm$  SEM. Significant difference between means was tested by Students t-test using GraphPad Prism software, version 5.0. Differences were considered statistically significant at  $p < 0.001$ .

## RESULTS

As Table 2 shows, the animals on HFD showed a more rapid increase in weight than control. Though not quantified, most of them showed varying degrees of hair loss. The weight gain was significantly higher for group II. The consumption of diet by the animals was 15 to 20 g/day.

**Table 2:** Mean body weight of rats

Group	Mean body weight (g)	
	Day 0	Day 120
I (Control)	214.3 $\pm$ 0.3	224.7 $\pm$ 0.4
II (HFD)	220.3 $\pm$ 0.4	265.3 $\pm$ 0.3

C-reactive protein and lipid profile data are shown in Table 3. It is clear from the data that in all the lipid determination, there was a marked difference between the group I (control) and group II (HFD) group. Total cholesterol increased about 5-fold from an average normal level in group II after 120 days. The LDL, VLDL Cholesterol showed significant difference, an increase of about 4-fold in group II as compared to control group I. Triglyceride concentration in HFD group II showed significant increase in its level. On the other hand, HDL cholesterol which is good cholesterol showed decrease in its level in group II. In this study, HFD group II showed marked increase in lipid profile but the concentration of C-reactive protein observed within normal range even after 120 days.

## DISCUSSION

Rats, which usually have omnivorous habits, but in which atherosclerosis has been experimentally induced, has been of great interest [16,17]. Induction of atherosclerosis in albino rat using a diet that included cholesterol, sodium cholate, and thiouracil has been reported by Fillios as well as by Hartroft and Thomas. The latter added a large amount of butter to the basic diet used by Fillos in their study and reported a high percentage of thrombosis and myocardial infarction in albino rats [18,19].

The development of atherosclerosis is a complex multi-factorial process involving a variety of environmental and genetic interactions. The data obtained in the present study allowed us to address the question of whether the lipid profile and the period of exposure to high fat diet are indeed important determinants of atherosclerotic lesion and whether c-reactive protein played a role. Our data show that exposure of the animals to high fat diet led to the development of atherosclerosis as evidenced by the lesion found in the intima and also the significantly increased serum lipid levels in group II (HFD) animals. However, the level of c-reactive protein in serum was within normal range. C-

**Table 3:** Serum C-reactive protein (CRP) and lipid profiles in the rats

Parameter (mg/dL)	Group I (Control)		Group II (HFD)	
	Day 0	Day 120	Day 0	Day 120
C-reactive protein ( $\times 10^{-2}$ )	2.67 $\pm$ 0.09	3.02 $\pm$ 0.06	2.78 $\pm$ 0.03	3.37 $\pm$ 0.15
Total cholesterol	54.2 $\pm$ 2.3	57.2 $\pm$ 2.9	57.3 $\pm$ 2.9	269.0 $\pm$ 3.6***
HDL cholesterol	40.8 $\pm$ 3.0	41.1 $\pm$ 1.5	37.7 $\pm$ 2.5	27.3 $\pm$ 1.1*
Triglycerides	108.3 $\pm$ 4.9	109.3 $\pm$ 5.0	112.5 $\pm$ 2.7	175.5 $\pm$ 4.0***
LDL cholesterol	35.0 $\pm$ 4.1	47.6 $\pm$ 2.3	109.2 $\pm$ 6.1	125.5 $\pm$ 3.8***
VLDL cholesterol	21.7 $\pm$ 1.0	20.7 $\pm$ 1.5	11.5 $\pm$ 0.6	53.8 $\pm$ 0.7***
TC/HDL ratio	1.34 $\pm$ 0.06	1.38 $\pm$ 0.04	1.6 $\pm$ 0.14	9.90 $\pm$ 0.29***
LDL/HDL ratio	0.84 $\pm$ 0.05	2.58 $\pm$ 0.20	2.95 $\pm$ .23	4.63 $\pm$ 0.24**

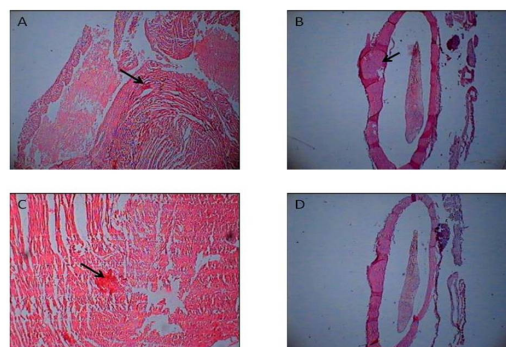
Values are expressed as mean  $\pm$  SEM, n = 12, p < 0.001

reactive protein has been recommended as the marker of choice to monitor cardiovascular risk, being a stronger predictor of atherosclerosis than even plasma LDL concentration. It is postulated that as an acute-phase protein, elevation of plasma CRP may signal underlying atherosclerosis process [20].

Fig 1A shows lipomatous changes in group II (HFD) animals, which consisted mostly of extracellular lipid infiltrations of the coronary vessels without proliferation. Fig 1B shows atheromatous lesion in the aorta of group II animals. In Fig 1C, the myocardial infarcts observed (group II animals) were small and irregular in outline. These infarcts were characterized by degeneration of myocardial fibers which are clearly separated from the rest of the myocardium. Fig 1D shows normal aorta (group I) with no cardiovascular lesions encountered.

Sun and colleagues used well established animal atherosclerosis models, i.e., both cholesterol fed and Watanabe heritable hyperlipidemic rabbits, to study the role of CRP in atherogenesis and generated elaborate results. First, CRP level was significantly elevated in hypercholesterolemic rabbits. Second, elevated CRP levels

strongly correlated with the extent of atherosclerosis in these animals [21]. These findings indicate the need for further investigation of human CRP concentration in cardiovascular disease.



**Fig 1:** Histopathology of heart shows (A) branch of coronary artery in a frozen section of heart with marked lipid infiltration in the wall, indicative of an early atheromatous type of vascular lesion (x 40); (B) section of aorta from a high fat diet rat stained with hematoxyline and eosin showing plaque development with foam cell (x 100); (C) myocardial infarct in heart (group II) with degeneration of myocardial fibers which are clearly separated from the rest of the myocardium (x 100); (D) section of aorta chow-fed diet rat (group I), stained with hematoxyline and eosin (x 100).

## CONCLUSION

We confirm that C-reactive protein did not play any role in the development of atherosclerosis in Wistar breed albino rat and therefore this animal model can't be used to extrapolate C-reactive protein data. However, this animal model would be useful for study of antihyperlipidemic drugs and atherosclerosis development.

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